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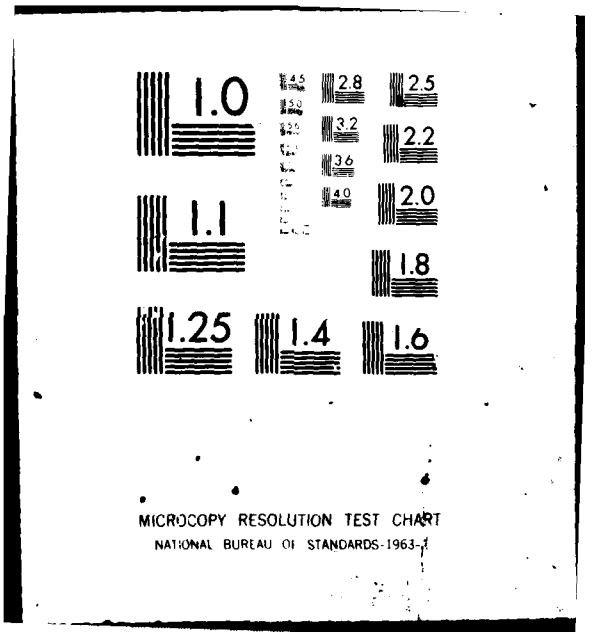
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Interim Report No. 2

Metabolic Disposition of Labeled WR-158,122 in a Bile Duct Cannulated Rhesus Monkey

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January 18, 1980

Revised February 18, 1980

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

Contract No. DAMD17-79-C-9106

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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1 REPORT NUMBER Interim Report No. 2	2 GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Metabolic Disposition of Labeled WR-158,122 in a Bile Duct Cannulated Rhesus Monkey		5 TYPE OF REPORT & PERIOD COVERED Interim Report No. 2 November 15-January 18, 1980 6. PERFORMING ORG. REPORT NUMBER Interim Report No. 2
7. AUTHOR(s) Carl C. Smith, Ph.D.		8. CONTRACT OR GRANT NUMBER(s) DAMD 17-79-C-9106
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Environmental Health and Department of Laboratory Animal Medicine, University of Cincinnati Col. of Med., Cincinnati, OH 45267		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 2192040 975-8119 612770.80300 2583 S18064
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command Fort Detrick, Frederick, Maryland 21701		12. REPORT DATE January 18, 1980 Revised February 18, 1980 13. NUMBER OF PAGES 14
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		15. SECURITY CLASS. (of this report) Unclassified 15a. DECLASSIFICATION DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited. The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

sulfonylquinazoline
WR-158,122
2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline
rhesus monkey
bile duct cannulation
blood levels
excretion

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A 4 kg female rhesus monkey with a bile duct cannula was administered two single oral doses of ^{14}C-labeled WR-158,122. The first dose was given 7 days after initial surgery and the second dose 9 days later. Part (approximately 50%) of the first dose was lost by vomiting but the entire second oral dose was retained. Following the first treatment total excretion of drug in the urine (13.1%) and feces (32.8%) was equivalent as ^{14}C to 45.9% of the dose, not corrected for loss from vomiting. An additional 4.12% was excreted in the

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bile of which about 75% was returned to the animal. Following the second 5 mg/kg oral dose excretion (as ^{14}C) in the urine (21.5%) and feces (72.8%) accounted for 94.3% of the dose. An additional 9.4% was excreted in the bile and again about 75% was returned. These data indicate that at least in this monkey WR-158,122 is moderately absorbed and to a limited degree excreted via the bile.

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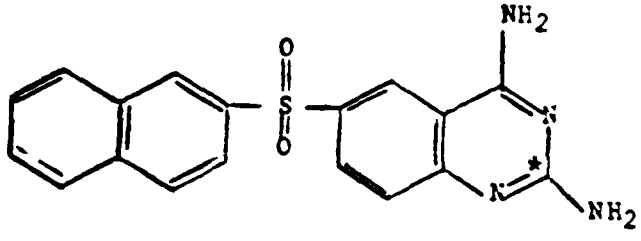
Studies on the metabolic fate of WR-158,122 [2,4-diamino-6-(2 naphthylsulfonyl)-quinazoline-2-^{14}C], the antifolate antimalarial compound shown below



*^{14}C

have been carried out in one bile duct cannulated rhesus monkey. Data on blood levels, plasma levels, and excretion in bile, urine, and feces are detailed in this report.

Materials and Methods

Compounds. WR-158,122 [2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline], empirical formula $C_{18}H_{14}SO_2N_4$, mol. wt. 350, was supplied by Walter Reed as Bottle AY 65859. The ^{14}C-preparation labeled in the 2 position was synthesized by Research Triangle Institute and was supplied as Lot No. 2572-110, dated 8/20/79, with a specific activity of 69 µCi/mg or 24 mCi/m mole. The radiochemical purity in a number of TLC systems was > 98%.

Treatment Suspension. The treatment suspension was prepared by grinding intimately 307.13 mg of cold drug and 3.08 mg of ^{14}C labeled drug in a glass mortar with a pestle, with small additions of diluent (0.2% methylcellulose and 0.4% Tween 80 in distilled water), until a smooth suspension was achieved. The suspension was decanted into a tared round-bottom polycarbonate 200 ml centrifuge bottle containing glass beads and diluted to 124.08 g. It contained 2.50 mg WR-158,122/ml and 1.86 µCi/ml. The suspension was stored at 4°C. Assay of the suspension gave the following results:

4,136,000 dpm/ml
1654 dpm/µg
1.86 µCi/ml

Analytical Procedures. Samples of blood, bile, urine and feces were collected as described in Interim Report No. 1 (November 20, 1979) and processed for liquid scintillation counting by the standard procedures of this laboratory as described in the same report.

Monkey. One 4 kg female rhesus monkey which had been acclimated to a primate chair was employed for this study. Water was available ad libitum

and the chair facilitated the collection of blood samples, collection of iced urine, feces, and bile.

The monkey's bile duct was cannulated on 12/4/79. The monkey was fasted for 24 hours prior to surgery. It was tranquilized with 0.25 ml Ketamine HCl, im, anesthetized with 1.5 ml sodium thiamylal, iv, and anesthesia maintained with methoxyfluorane.

A midline incision was used to expose the gall bladder and the common bile duct. A two centimeter length of latex tubing was double ligated over the common bile duct. A Bard latex T-tube (size 8 or 10) was installed into the fundus of the gall bladder. The exteriorized Bard T-tube was elongated by attaching a size No. 8 feeding tube. This tube delivered bile into a condom which was supported on a part of the monkey chair. The monkey could move freely without putting strain on the exteriorized cannula.

A second Bard T-tube (size 8 or 10) implanted in the duodenum permitted the administration of bile, liquid nutriments, or water.

On 12/8/79 the monkey escaped from the chair and pulled out the bile cannula. Corrective surgery was immediately performed and the bile cannula was re-installed, after which the monkey's recovery was uneventful.

On 12/11/79 the monkey was administered a single oral dose of WR-158,122 ^{14}C (5 mg/kg) via a No. 8 French nasogastric tube. The monkey vomited two times within five hours after this treatment so that a substantial portion of the dose was lost. Blood samples were collected from the right or left antecubital vein at 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours. Bile, urine and feces samples were collected at approximately the same times, as indicated in the tables. Each bile sample was weighed, ca 25% frozen for analysis and the remainder returned to the monkey.

On the 9th day following the first treatment, this monkey was given a second dose of WR-158,122 ^{14}C (5 mg/kg) by soft oral catheter. This dose was retained. Blood, bile, urine and feces samples were collected for the same time intervals as was done following the first treatment.

Two days after the second dose the monkey developed small decubitus ulcers on each elbow. For six days the monkey received 1 ml injections of Diathal®*, im, in addition to topical application of BFI®** powder and nitrofurazone*** ointment.

SMA 12/60 assays were run on plasma samples at 1 day after surgery, just prior to the first and second drug treatments and on the seventh day after the second treatment.

Results

Blood Levels. Study of blood and plasma levels of radioactivity for the first treatment period shows that the peak levels occurred at about 8 hr. Detectable levels of the drug as ^{14}C in blood and plasma persisted through 72 hr(see Table 1). There was a marked decline in hematocrit levels from 24 hr (41%) through 72 hr (33%).

* Diathal® - Each ml contains 200,000 units procaine penicillin G, 250 mg dihydrostreptomycin, 10 mg chlorpheniramine maleate, and 25 mg diphemanil-methylsulfate. Preservatives are 20 mg procaine HCl, 8.8 mg sodium citrate, 10 mg lecithin (with 2% tricalcium phosphate) 1.5 mg methyl paraben and 0.5 mg propyl paraben as preservative. Water for injection q.s. Schering Corp., Kenilworth, NJ 07033.

** BFI® powder - Antiseptic first-aid powder. Contains Bismuth-formic-iodide, Zinc phenolsulfonate, Bismuth Subgallate, Amol (mono-n-amyl hydroquinone ether), Potassium Alum, Boric Acid, Menthol, Eucalyptol, Thymol and inert diluents. Calgon Consumer Products Company, Inc., Subsidiary of Merck & Co., Inc., Pittsburgh, PA 15230.

*** Nitrofurazone Ointment, N.F. - Topical Antibacterial Ointment (for external use only, not for ophthalmic use) distributed by Henry Schein, Inc., Port Washington, NY 11050.

Following the second treatment the peak for blood and plasma levels occurred at about 4 hr. Again the decline in hematocrit levels from 2 hr (37%) to 72 hr (29%) was marked.

Bile Excretion. Excretion of bile following the first treatment was not as abundant as that which followed the second treatment (see Table 2). During the 24 hours following the first treatment only 20.5 g of bile were excreted, whereas in the 24 hr following the second treatment 60.1 g of bile were excreted. Peak (2.2%) bile excretion occurred during the 48-72 hr interval following the first treatment, whereas the peak (7.8%) for the second treatment was a broad peak covering 0-12 hr.

From 24 to 48 hr following the first treatment only 19.2 g of bile were excreted. In contrast, during the same period following the second treatment, 88 g of bile were excreted.

The two vomiting episodes which occurred at 4 and 5 hours after the first treatment obviously reduced the amount of drug available for absorption and the delayed excretion pattern after the first dose may have been related to these.

Total radioactivity in the bile withheld (ca 25% of the total) amounted to 1.09% in first treatment and 2.63% for the second. The bile was not returned on a strict schedule. Frequently it was administered quite some time after the monkey's morning feeding. The bile samples were usually dark green and of low viscosity.

When the monkey was not eating satisfactorily on the second day of the first treatment period, a liquid nutriment called Esbilac® was administered

*Esbilac® - Skim milk, water, vegetable oil, casein, egg yolk, calcium carbonate precipitated, potassium phosphate monobasic, lecithin, calcium hydroxide, choline chloride, sodium bicarbonate, potassium chloride, carrageenan, salt, potassium phosphate, dibasic, magnesium carbonate, magnesium sulfate, vitamin A and vitamin E supplement, iron sulfate, zinc sulfate, niacin supplement, calcium pantothenate, copper sulfate, vitamin B_{12} supplement, vitamin D_3 supplement, manganese sulfate, riboflavin, thiamin HCl, pyridoxine HCl, potassium iodide. Borden Chemical, Borden Inc., P. O. Box 419, Norfolk, VA 23501 and Borden International, 420 Lexington Avenue, New York, NY 10017.

in two 20 ml infusions by duodenal cannula.

Urinary and Fecal Excretion. Following the first treatment the monkey excreted 13.1% of the dose in urine and 32.8% in feces giving a combined urinary and fecal excretion of 45.9% (as ^{14}C) (see Table 3). The peak for urinary excretion following this treatment was 7.6% of the dose during the 48-72 hr period. Tne peak for fecal excretion was 20.1% and occurred during the 24-48 hr period.

Following the second treatment the monkey excreted 21.5% of the dose in urine and 72.8% of the dose in the feces. Combined urinary and fecal excretion totaled 94.3%. It is noteworthy that 82% of the total urinary excretion was excreted during the 0-12 hr period.

The monkey was active and exhibited generally a good appetite for food (monkey chow with some fruit supplements) and water. The antibiotic therapy for six days did not appear to complicate the second study. Bile flow, fecal excretion and urine excretion were very good throughout the treatment period and for the following week.

The SMA 12/60 assays showed moderately elevated SGOT and LDH and appreciably elevated alkaline phophatase values. We had similar observations in a number of bile duct cannulated monkeys studied earlier (1969-1972).

Discussion

The analysis of the first treatment of this bile duct cannulated monkey was seriously impaired by the vomiting episodes at 4 and 5 hr after dosing. From the total recovery of radioactivity in bile, urine, and feces (47%; Table 3) we concluded that about 50% of the dose was lost. The differences in the blood level curves and urinary excretion patterns suggested that absorption of the retained portion of the first dose was delayed.

When blood levels after the second treatment are compared to blood levels we observed previously in normal monkeys given the same dose of

WR-158,122^{14}C we find that the 2, 4, and 6 hr levels are very close to the mean blood levels of the normal monkeys (C. C. Smith et al., Interim Report No. 40-1, March 16, 1972; Contract No. DADA 17-67-C-7065). The 8, 12, 24, 48, and 72 hr blood levels after the second treatment are appreciably lower than the mean values for these same time periods in these normal monkeys. However, when the range for the 6 normal monkeys is considered, the levels we observed for these time periods in this bile duct cannulated monkey are close to the low figures for normal monkeys.

As in the studies of the 6 normal monkeys we find that plasma levels in the initial 2-24 hr period are appreciably higher than levels in whole blood. After 24 hr plasma levels are somewhat lower than whole blood levels.

The total excretion of 9.4% in bile during the second treatment indicates that this compound was moderately absorbed and to a limited degree excreted via the bile and urine. Eighty-three percent of the total radioactivity excreted in the bile (second treatment) was excreted by 12 hr. Biliary excretion beyond this time was relatively insignificant.

Further evidence that maximum absorption of the compound occurred in the 0-12 hr period (second dose) is the finding that 82% of the 21.5% excreted in the urine also was excreted in 0-12 hr. Only 3.9% of the dose was excreted in the 24-144 hr time period.

Urinary excretion in this bile duct cannulated monkey was higher than the mean observed for 6 normal monkeys (ibid), but almost identical urinary excretion was observed in one of the normal monkeys.

Most of the drug was excreted in the feces (i.e. 72.8%; second treatment) and primarily during the 24-48 hr period. Excretion via this route was practically complete by 72 hr. In our earlier studies of normal monkeys, the mean fecal excretion in 4 of the 6 normal animals accounted for 70.1% of the dose. Thus, fecal excretion in this bile duct cannulated monkey compared

favorably with observations on 4 normal rhesus monkeys studied seven years earlier.

Thus, from these limited data it appears that this antimalarial compound is moderately absorbed and to a limited degree excreted via the bile.

Summary

1. A 4 kg female rhesus monkey with a bile duct cannula was administered two single oral doses of ^{14}C-labeled WR-158,122. The first dose was given 7 days after initial surgery and the second dose 9 days later. Part (approximately 50%) of the first dose was lost by vomiting but the entire second oral dose was retained.

2. Following the first treatment total excretion of drug in the urine (13.1%) and feces (32.8%) was equivalent (as ^{14}C) to 45.9% of the dose, not corrected for loss from vomiting. An additional 4.12% was excreted in the bile of which about 75% was returned to the animal.

3. Following the second 5 mg/kg oral dose, excretion (as ^{14}C) in the urine (21.5%) and feces (72.8%) accounted for 94.3% of the dose. An additional 9.4% was excreted in the bile and again about 75% was returned.

4. These data indicate that at least in this monkey, WR-158,122 (as ^{14}C) is moderately absorbed and to a limited degree excreted via the bile.

Table 1

Blood and Plasma Levels of WR-158,122 (as ^{14}C) in a Bile Duct Cannulated Monkey

Single Oral Doses of 5 mg/kg

Hours Post Dose	First Treatment* µg/g (as ^{14}C) Blood	Plasma	Hct	Second Treatment µg/g (as ^{14}C) Blood	Plasma	Hct
2	0.04	0.06	40	1.41	2.24	37
4	0.05	0.08	36	1.91	2.60	31
6	0.04	0.07	35	0.83	1.23	33
8	0.06	0.10	41	0.45	0.70	32
12	0.04	0.06	40	0.18	0.27	32
24	0.05	0.09	41	0.06	0.08	32
48	**	0.05	39	0.02	0.01	27
72	0.03	0.04	33	0.01	<0.01	29

* Vomited part of dose.

** Contaminated sample.

Table 2

Biliary Excretion of WR-158,122 (as ^{14}C) in a Bile Duct Cannulated Monkey

Single Oral Doses of 5 mg/kg

First Treatment*				Second Treatment			
Hours Post Dose	Percent Dose Period	Percent Dose Total	Wt. in grams	Hours Post Dose	Percent Dose Period	Percent Dose Total	Wt. in grams
0-6	0.04	0.04	2.6	0-6	3.9	3.9	14.2
6-12	0.36	0.40	15.4	6-12	3.9	7.8	21.4
12-24	0.17	0.57	2.5	12-24	0.91	8.71	24.5
24-48	1.2	1.77	19.2	24-48	0.55	9.26	88.0
48-72	2.2	3.97	36.8	48-72	0.07	9.33	63.4
72-96	0.11	4.08	48.8	72-96	0.02	9.35	73.0
96-120	0.02	4.10	27.9	96-120	0.01	9.36	75.0
120-144	0.01	4.11	26.3	120-144	<0.01	9.36	51.7
144-168	0.01	4.12	57.9	144-168	<0.01	9.36	76.0

*Vomited part of dose.

Table 3

Cumulative Excretion of WR-158,122 (as ^{14}C) in Bile, Urine and Feces from a Bile Duct Cannulated Monkey

Single Oral Doses of 5 mg/kg

First Treatment**

Excretion in Percent of Dose

Hours Post Dose	Bile*		Urine		Feces		Combined
	Period	Total	Period	Total	Period	Total	Total
0-12	0.12	0.12	0.80	0.80			0.92
12-24	0.17	0.29	0.86	1.7	0.20	0.20	2.2
24-48	0.30	0.59	3.1	4.8	20.1	20.3	25.7
48-72	0.48	1.07	7.6	12.4	3.9	24.2	37.7
72-96	0.02	1.09	0.48	12.9	7.2	31.4	45.4
96-120	<0.01	1.09	0.13	13.0	0.92	32.3	46.4
120-144	<0.01	1.09	0.05	13.1	0.46	32.8	47.0
144-168	<0.01	1.09	0.04	13.1	N.A.***	32.8	47.0
Second Treatment							
0-12	2.3	2.3	17.6	17.6			19.9
12-24	0.21	2.5	1.1	18.7	6.5	6.5	27.7
24-48	0.11	2.6	2.1	20.8	56.1	62.6	86.0
48-72	0.01	2.6	0.36	21.2	5.9	68.5	92.3
72-96	<0.01	2.6	0.13	21.3	3.7	72.2	96.1
96-120	<0.01	2.6	0.06	21.4	0.25	72.5	96.5
120-144	<0.01	2.6	0.07	21.5	0.32	72.8	96.9
144-168	<0.01	2.6	<0.01	21.5	<0.01	72.8	96.9

* Figures represent only portion saved for analysis
** Vomited part of dose
***Not assayed

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January 18, 1980; Revised February 18, 1980

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